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(57) Abstract

A method for promoting the growth or recovery of a selected organ in a mammal. A nutritional formula containing a source of dietary protein in a form for increasing the protein concentration or rate of protein synthesis in the selected organ, is administered to the patient. The dietary protein is in the form of protein hydrolysate, a peptide mixture isolated from protein hydrolysates, or free amino acids, or combinations thereof.

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Organ Specific Nutrition

Field of the Invention

This invention relates to a method of providing nutrition to a patient for causing a desired effect in a selected organ; for example increasing the protein concentration or rate of protein synthesis in the organ.

Background to the Invention

Nutritional formulas based upon naturally occurring proteins are well known in infant nutrition and clinical nutrition; especially formulas based upon milk and soy proteins. Further, hydrolysates of these proteins are commonly used in infant nutrition and clinical nutrition; particularly in hypoallergenic formulas and formulas for patients suffering from various intestinal absorption problems. Also, hydrolysates are commonly used in clinical nutrition due to their reduced tendency, as compared to proteins, to coagulate. It is also known to use free amino acids in nutritional formulas; either alone or in combination with protein or protein hydrolysates. Usually free amino acids are used for patients suffering from particular diseases or conditions such as inflammatory bowel disease, intractable diarrhoea, short bowel syndrome, and the like.

In all cases, the protein, hydrolysates or free amino acids in the nutritional formulas are intended to provide a source of amino acids to meet the general amino acid needs of the patient. Whether protein, hydrolysate or free amino acid, or a mixture of any of these is used, usually depends upon the condition of the intended consumer. If the intended consumer is a normal, healthy person, whole dietary protein is generally used. However, if the person suffers from a particular disease or condition, or is allergic to whole protein or is at risk of developing an allergy, a hydrolysate or free amino acid mix which the person is better able to tolerate or absorb is generally used.

There is also an interest in using protein hydrolysates in nutrition since it is generally accepted that protein hydrolysates are more rapidly absorbed in the intestine than whole protein or free amino acids (Rerat A.A. ; 1993; Proceedings of the Nutrition Society, 52, 335-344). However, it is not clear whether this faster absorption translates into better nitrogen utilisation since studies carried out to date have provided conflicting results (Collin-Vidal *et al*; 1994; Endocrinol. Metab., 30, E 907-914). Further, this interest is in the sense of

providing a source of amino acids to meet the general amino acid needs of the patient and not to specifically provide for the needs of individual organs.

However, in many instances, a person may be suffering from organ specific problems such as depletion of cells, incomplete or improper functioning, underdevelopment or fatigue, in the organs. Providing nutrition to the person in general sense, although beneficial, does not address these organ specific problems.

Various naturally-occurring and synthetic peptides have been reported in the literature as being useful for stimulating the growth of specific tissue. For example, international patent publication WO 92/20707 discloses certain bombesin analogs which may be used to stimulate or antagonise growth of lung tissue. Bombesin itself is a peptide of 14 amino acids which is isolated from the skin of the frog Bombina Bombina. As another example, European patent 0017867 discloses the use of a hydrolysate of a blood plasma extract as an agent for increasing liver growth. Other synthetic peptides for increasing liver growth are disclosed in Pickart *et al*; 1973; Biochem. Biophys. Res. Commun. 54(2), 562-6 (Gly-His-Lys); and Japanese patent application 05-229940 (di- and tri-peptides containing Ala and Gln). However these peptides are not considered dietary protein and it is not practicable to use them as the primary protein source in nutritional formulas.

Summary of the Invention

It has now been discovered that the form in which dietary protein is provided has a selective effect on the protein concentration, RNA concentration, ribosomal efficacy, and rate of protein synthesis in different organs. This offers the advantage of being able to promote the growth or recovery of a particular organ by providing the dietary protein in a form which increases the protein concentration or the rate of protein synthesis, or both, in that organ.

Accordingly, on one aspect, this invention provides a method for promoting the growth or recovery of a selected organ in a mammal, the method comprising administering to the mammal a nutritional formula containing a source of dietary protein in a form for increasing the protein concentration or rate of protein synthesis in the selected organ.

Without wishing to be bound by theory, it is believed that protein hydrolysates having a higher degree of hydrolysis are rapidly digested and

absorbed in the upper small intestine. Therefore protein substrate is available for protein synthesis in the upper small intestine. In this way, the upper small intestine may be targeted. Intact protein and protein hydrolysates having a lower degree of hydrolysis take longer to digest and are more slowly absorbed in the lower small intestine. Therefore protein substrate is available for protein synthesis in the lower small intestine. Also, the lower rate of absorption may result in more protein substrate being available for protein synthesis in the muscles due to the decrease in liver oxidation. In this way, the lower small intestine and muscles may be targeted.

In another aspect, this invention provides the use of a selected form of dietary protein which increases protein concentration or rate of protein synthesis in a selected organ as a protein source in the preparation of a nutritional formula for promoting the growth or recovery of the selected organ in a mammal.

Preferably the source of dietary protein is in the form of a protein hydrolysate, or free amino acids, and combinations thereof. Preferably the protein hydrolysates are hydrolysates of milk protein or a peptide mixture isolated from hydrolysates of milk protein.

In one specific embodiment, this invention provides a method for increasing protein concentration and synthesis in the small intestine, the method comprising administering to a patient an effective amount of a nutritional formula containing a protein hydrolysate having a degree of hydrolysis of at least about 30%. Preferably the protein hydrolysate comprises more than about 30% by weight of di- and tri-peptides. Further, the protein hydrolysate preferably has a non protein nitrogen concentration of at least about 85% of total nitrogen.

Preferably, the method may be used to treat patients suffering from illnesses or damage to the duodenum and jejunum and to promote recovery of the duodenum and jejunum. The method is more preferably used to treat patients suffering from illnesses or damage to the duodenum and to promote recovery of the duodenum.

In another specific embodiment, this invention provides a method for increasing protein concentration and synthesis in the jejunum, the method comprising administering to a patient an effective amount of a nutritional formula containing a protein source in the form of (i) a protein hydrolysate having a degree of hydrolysis of at least about 15%; (ii) free amino acids; or (iii) mixtures thereof. Preferably the protein hydrolysate comprises more than about 20% by weight of di- and tri-peptides. Further, the protein hydrolysate

preferably has a non protein nitrogen concentration of at least about 60% of total nitrogen.

Preferably, the method may be used to treat patients suffering from illnesses or damage to the jejunum to promote recovery of the jejunum.

In another aspect, this invention provides a method for the treatment of a patient suffering from illness of or damage to the duodenum, the method comprising (i) administering to a patient an effective amount of a nutritional formula containing a protein hydrolysate having a degree of hydrolysis of at least about 30% to initiate recovery of the duodenum; and (ii) thereafter administering to a patient a nutritional formula containing dietary protein in the form of whole protein or a hydrolysate having a degree of hydrolysis less than about 15%.

In another aspect, this invention provides a method for maintaining muscle protein synthesis and for the prophylaxis or treatment of muscular atrophy, the method comprising administering a patient an effective amount of a nutritional formula containing dietary protein in the form of whole protein, a hydrolysate having a degree of hydrolysis less than about 15%, or free amino acids, or a combination thereof.

The patient may have a compromised gut function and is preferably administered a nutritional formula containing dietary protein in the form of free amino acids.

In another aspect, this invention provides a nutritional formula for increasing protein concentration and synthesis in the intestine, the nutritional formula comprising: a source of dietary protein in the form of a protein hydrolysate having a degree of hydrolysis of at least about 30%, a non protein nitrogen concentration of at least about 85% of total nitrogen, and comprising more than about 30% by weight of di- and tri-peptides; a carbohydrate source; and a lipid source.

Preferably at least about 60% of the components of the protein hydrolysate have a molecular weight less than about 367; more preferably at least about 50% of the components of the protein hydrolysate have a molecular weight of about 127 to about 367.

The nutritional formula is preferably an enterally administrable formula; for example in the form of a powder, a liquid concentrate, or a ready-to-drink beverage.

Embodiments of the invention are now described by way of example only.

Detailed Description of Preferred Embodiments of the Invention

In this specification, the term "degree of hydrolysis" (DH) means the percentage of nitrogen in the form of amino nitrogen as compared to total nitrogen. It is a measure of the extent to which the protein has been hydrolysed.

It has been discovered that the form in which dietary protein is consumed influences various organs to different extents. This provides the significant advantage that the growth or recovery of a specific organ may be selectively enhanced through nutrition. All that is required is to specifically enhance the growth or recovery of an organ is administer a nutritional formula which contains the dietary protein in the correct form.

The dietary protein which is used may be any suitable dietary protein; for example animal proteins (such as milk proteins, meat proteins and egg proteins); vegetable proteins (such as soy protein, wheat protein, rice protein, and pea protein); mixtures of free amino acids; or combinations thereof. Milk proteins such as casein and whey protein are particularly preferred.

For targeting nutrition to the small intestine, one suitable form of the dietary protein is protein hydrolysate. Casein and whey protein hydrolysates are preferred. The extent to which the protein is hydrolysed influences the area of the intestine in which the protein is digested and used for protein synthesis.

In particular, hydrolysates having a degree of hydrolysis of about 10% to about 15%, are found to increase relative weight of the liver as compared to free amino acid mixes. Further, these hydrolysates may be used for maintaining muscle protein synthesis. Hydrolysates having a degree of hydrolysis of about 15% to about 25% are found to increase the concentration of protein in the jejunum, the relative weight of the jejunum and the rate of protein synthesis in the jejunum. Highly hydrolysed protein which has a degree of hydrolysis of greater than 25% or which contains more than 25% by weight of di- and tri-peptides, more preferably greater than 30%, is found to increase the rate of protein synthesis in the jejunum and the duodenum; particularly the duodenum.

The protein hydrolysates may be produced using procedures which are well known in the art or may be obtained commercially. For example, nutritional formulas containing hydrolysates having a degree of hydrolysis less than about 15% are commercially available from Nestlé Nutrition Company under the trade mark Peptamen®. Hydrolysates having a degree of hydrolysis above about 15% may be prepared using the procedure described in EP 0322589.

The dietary protein may also be in the form of a mix of free amino acids; preferably such that the mix provides a balanced amino acid profile. Dietary protein in the form of a mix of free amino acids is found to increase the relative weight of the jejunum and the rate of protein synthesis in the jejunum. Further, free amino acids maintain levels of protein synthesis in the muscles while being in an easily digestible form. Hence free amino acids are suitable for maintaining muscle protein synthesis in patients having compromised gut function.

It is also possible to provide the dietary protein in a variety of forms such that several organs are simultaneously provided specific nutrition.

The source of dietary protein preferably provides about 5% to about 30% of the energy of the nutritional formula; for example about 10% to about 20% of the energy. The remaining energy of the nutritional formula may be provided in the form of carbohydrates and fats.

If the nutritional formula includes a fat source, the fat source preferably provides about 5% to about 55% of the energy of the nutritional formula; for example about 20% to about 50% of the energy. The lipids making up the fat source may be any suitable fat or fat mixture. Vegetable fats are particularly suitable; for example soy oil, palm oil, coconut oil, safflower oil, sunflower oil, corn oil, canola oil, lecithins, and the like. Animal fats such as milk fats may also be added if desired. The lipids may also include medium-chain triglycerides; for example up to about 80% by weight of lipids as medium-chain triglycerides. Fractionated coconut oil is a suitable source of medium-chain triglycerides.

If the nutritional formula includes a carbohydrate source, the carbohydrate source preferably provides about 40% to about 80% of the energy of the nutritional formula. Any suitable carbohydrates may be used, for example sucrose, lactose, glucose, fructose, corn syrup solids, and maltodextrins, and mixtures thereof.

Dietary fibre may also be added if desired. Numerous types of dietary fibre are available. Suitable sources of dietary fibre, among others, include soy, pea, oat, pectin, guar gum, and gum arabic. If used, the dietary fibre preferably comprises up to about 5% of the weight of the nutritional formula.

Suitable vitamins and minerals may be included in the nutritional formula in the usual manner to meet the appropriate guidelines.

One or more food grade emulsifiers may be incorporated into the nutritional formula if desired; for example diacetyl tartaric acid esters of mono-diglycerides, lecithin and mono- and di-glycerides. Similarly suitable salts and stabilisers may be included.

The nutritional formula may be prepared in any suitable manner. For example, the nutritional formula may be prepared by blending together the source of dietary protein, the carbohydrate source, and the fat source in appropriate proportions. If used, the emulsifiers may be included in the blend. The vitamins and minerals may be added at this point but are usually added later to avoid thermal degradation. Any lipophilic vitamins, emulsifiers and the like may be dissolved into the fat source prior to blending. Water, preferably water which has been subjected to reverse osmosis, may then be mixed in to form a liquid mixture. The temperature of the water is conveniently about 50°C to about 80°C to aid dispersal of the ingredients. Commercially available liquefiers may be used to form the liquid mixture. The liquid mixture is then homogenised; for example in two stages.

The liquid mixture may then be thermally treated to reduce bacterial loads. For example, the liquid mixture may be rapidly heated to a temperature in the range of about 80°C to about 150°C for about 5 seconds to about 5 minutes. This may be carried out by steam injection, autoclave or by heat exchanger; for example a plate heat exchanger.

The liquid mixture may then be cooled to about 60°C to about 85°C; for example by flash cooling. The liquid mixture may then be again homogenised; for example in two stages at about 7 MPa to about 40 MPa in the first stage and about 2 MPa to about 14 MPa in the second stage. The homogenised mixture may then be further cooled to add any heat sensitive components; such as vitamins and minerals. The pH and solids content of the homogenised mixture is conveniently standardised at this point.

If it is desired to produce a powdered nutritional formula, the homogenised mixture is transferred to a suitable drying apparatus such as a spray drier or freeze drier and converted to powder. The powder should have a moisture content of less than about 5% by weight.

If it is desired to produce a liquid formula, the homogenised mixture is preferably aseptically filled into suitable containers. Aseptic filling of the containers may be carried out by pre-heating the homogenised mixture (for example to about 75 to 85°C) and then injecting steam into the homogenised mixture to raise the temperature to about 140 to 160°C; for example at about 150°C. The homogenised mixture may then be cooled, for example by flash cooling, to a temperature of about 75 to 85°C. The homogenised mixture may then be homogenised, further cooled to about room temperature and filled into

containers. Suitable apparatus for carrying out aseptic filling of this nature is commercially available. The liquid formula may be in the form of a ready to feed formula having a solids content of about 10 to about 14% by weight or may be in the form of a concentrate; usually of solids content of about 20 to about 26% by weight. Flavours may be added to the liquid formulas so that the formulas are provided in the form of convenient, flavoursome, ready-to-drink beverages.

The nutritional formula may be used in the prophylaxis or treatment of a variety of conditions or diseased states. For example, many premature babies suffer from undeveloped intestines. A nutritional formula containing dietary protein in the form of hydrolysed protein or free amino acids may be fed to the babies to promote an increase in protein concentration and protein synthesis in intestinal tissue and hence increase development of the intestine. As a further example, many elderly people suffer from atrophy of the intestine. Hence these nutritional formulas may be fed to the elderly for the prophylaxis or treatment of intestinal atrophy.

Further, nutritional formulas containing dietary protein in the form of hydrolysed protein or free amino acids may be fed to patients who suffer from illnesses or damage to the intestine to promote recovery of the intestine; for example, inflammatory conditions of the gastro-intestinal tract (such as Crohn's disease and sepsis), gut epithelial damage, consequences of severe diarrhoea, post antibiotic colitis, gut surgery, jejunum resection, and atrophy after parenteral feeding.

The nutritional formulas may also be used to assist in the recovery of muscles after exercise of in the prophylaxis or treatment of muscular atrophy in the elderly. In this case, the source of dietary protein may be in the form of free amino acids, intact protein, or a hydrolysate having a degree of hydrolysis of less than about 15%.

The amount of the nutritional formula to be administered will vary depending upon the age of the patient, the condition or disease, and the severity of the condition or disease. However the amount may be readily set by a medical practitioner. In general, an amount of the nutritional formula sufficient to provide a daily dosage of dietary protein of about 3 g to about 300 g. A daily dosage of dietary protein of about 50 g to about 150 g is preferred for clinical applications.

The nutritional formula may be taken in multiple doses, for example 2 to 10 times a day, to make up the selected daily dosage or may taken in a single dose. If in a single dose form, the nutritional formula is conveniently taken in replacement of a meal. In the case of multiple doses, the nutritional formula is conveniently in the form of a convenience food; for example a ready-to-drink beverage. The nutritional formula may also be administered continuously by means of nasogastric tubes or enteral tubes such as jejunum tubes.

Example 1

a) Whole proteins

An amount of 5 kg of whey protein (obtained from Meggle GmbH under the trade name Globulal 80) is dispersed in demineralised water at 55°C to obtain protein concentration (N*6.38) of 10% by weight. The pH of the dispersion is adjusted by the addition of 190 g of calcium hydroxide and the dispersion is cooled to room temperature. The proteins are then dried by lyophilisation and packaged into metal cans.

The whole proteins have a degree of hydrolysis of about 4.41% and a non protein nitrogen concentration of about 1.1% on the basis of total nitrogen.

b) Hydrolysate 1

An amount of 6.25 kg of whey protein (obtained from Meggle GmbH under the trade name Globulal 80) is dispersed in 50 litres of demineralised water at 55°C. The pH of the dispersion is adjusted to 8.2 by the addition of 1.8 litres of 2M $Ca(OH)_2$. The proteins are then hydrolysed using 30 g of trypsin (Salt free pancreatic trypsin which has an activity of 6.8 AU/g and a chymotrypsin content of less than 5% and which is obtainable from Novo Nordisk Ferment AG, Dittigen, Switzerland). The hydrolysis reaction is continued for 4 hours at 55°C. During the reaction, the pH is regulated to 7.4 by the addition of 1.6N NaOH and 0.4N KOH. The enzymes are then inactivated by heating the reaction mixture to 80°C and holding the mixture at this temperature for about 5 minutes. The mixture is then cooled to 16°C. The hydrolysed proteins are then dried by lyophilisation and packaged into metal cans. A sample of the hydrolysed

proteins is then subjected to HPLC gel filtration to determine the molecular weight distribution.

Molecular weight	Percentage	Cumulative percentage
33730	0	0
25820	2.3	2.3
19860	4.3	6.6
15210	5.5	12.1
11670	6.9	19.0
8933	3.9	22.9
6855	3.6	26.5
5248	3.1	29.6
4027	3.7	33.3
3083	4.5	37.8
2366	9.4	47.2
1811	6.9	54.1
1387	6.9	61.0
1064	3.9	64.9
815	9.2	74.2
625	6.7	80.9
479	1.6	82.5
367	1.5	84.0
281	3.6	87.6
215	8.4	96.0
165	3.4	99.4
127	0.4	99.8
97	0.1	99.9
74	0.1	100.0

The hydrolysate has a degree of hydrolysis of about 14% and a non protein nitrogen concentration of about 54.5% on the basis of total nitrogen.

c) Hydrolysate 2

An amount of 6.25 kg of whey protein (obtained from Meggle GmbH under the trade name Globulal 80) is dispersed in 50 litres of demineralised water at 55°C. The pH of the dispersion is adjusted to 7.5 by the addition of 1.6 litres of 1M $Ca(OH)_2$ and 162 ml of a solution of 1.6M NaOH and 0.4M KOH. The proteins are then hydrolysed using 50 g of trypsin (obtainable from Novo Nordisk Ferment AG). The hydrolysis reaction is continued for 4 hours at 55°C. During the reaction, the pH is regulated to 7.4 by the addition of 1.6N NaOH and 0.4N KOH. The enzymes are then inactivated and non-hydrolysed protein is denatured, by heating the reaction mixture to 90°C and holding the mixture at this temperature for about 5 minutes.

The mixture is then cooled to 56°C and hydrolysed again for 1 hour using 50g of trypsin at 55°C. During the reaction, the pH is regulated to 7.4 by the addition of 1.6N NaOH and 0.4N KOH. The enzymes are then inactivated by heating the reaction mixture to 80°C and holding the mixture at this temperature for about 5 minutes. The mixture is then cooled to 18°C. The hydrolysed proteins are then dried by lyophilisation and packaged into metal cans.

A sample of the hydrolysed proteins is then subjected to HPLC gel filtration to determine the molecular weight distribution.

Molecular weight	Percentage	Cumulative percentage
33730	1.0	1.0
25820	2.7	3.7
19860	3.0	6.7
15240	2.8	9.5
11670	3.2	12.7
8954	2.1	14.8
6855	2.2	16.9
5260	2.3	19.2
4027	3.0	22.2
3090	3.8	26.0
2366	9.7	35.7
1811	7.0	42.7

1390	7.5	50.2
1064	4.2	54.4
817	10.4	64.8
625	8.7	73.5
480	2.1	75.6
367	1.7	77.3
282	5.2	82.5
216	11.1	93.6
165	4.9	98.5
127	0.8	99.3
97	0.2	99.5
74	0.3	99.8
57	0.2	100.0

The hydrolysate has a degree of hydrolysis of about 17.3% and a non protein nitrogen concentration of about 65.9% on the basis of total nitrogen.

d) Hydrolysate 3

An amount of 6.25 kg of whey protein (obtained from Meggle GmbH under the trade name Globulal 80) is dispersed in 50 litres of demineralised water at 55°C. The pH of the dispersion is adjusted to 7.5 by the addition of 1.6 litres of 1M $Ca(OH)_2$ and 162 ml of a solution of 1.6M NaOH and 0.4M KOH. The proteins are then hydrolysed using 250 g of Alcalase 2.4L (EC 940459 - obtainable from Novo Nordisk Ferment AG). The hydrolysis reaction is continued for 4 hours at 55°C. For the first hour of the reaction, the pH is regulated to 7.6 by the addition of 1.6N NaOH and 0.4N KOH.

An amount of 250g of Neutrase 0.5L (obtainable from Novo Nordisk Ferment AG) is added and the proteins are further hydrolysed for 4 hours at 50°C. The enzymes are then inactivated by heating the reaction mixture to 90°C and holding the mixture at this temperature for about 5 minutes. The reaction mixture is then cooled to 55°C.

The pH of the reaction mixture is adjusted to 7.33 by the addition of 1.6N NaOH and 0.4N KOH and the reaction mixture hydrolysed again for 4 hours using 100g of pancreatin at 55°C. During the reaction, the pH is regulated to 7.5

by the addition of 1M NaOH. The enzymes are then inactivated by heating the reaction mixture to 90°C and holding the mixture at this temperature for about 5 minutes. The mixture is then cooled to 4°C. The hydrolysed proteins are then dried by lyophilisation and packaged into metal cans.

A sample of the hydrolysed proteins is then subjected to HPLC gel filtration to determine the molecular weight distribution.

Molecular weight (Dalton)	Percentage	Cumulative percentage
5248	0.1	0.1
3083	0.1	0.2
2366	0.9	1.1
1811	1.2	2.3
1387	3.0	5.3
1064	1.4	6.7
815	6.7	13.4
625	14.2	27.6
479	5.3	32.9
367	1.6	34.5
281	10.3	44.8
215	22.2	67.0
165	14.5	82.4
127	6.8	89.2
97	10.5	99.7
74	0.2	99.9
57	0.1	100.00

The hydrolysate has a degree of hydrolysis of about 35% and a non protein nitrogen concentration of about 92.6% on the basis of total nitrogen. The hydrolysate is rich in small peptides, especially di- and tri-peptides which have a molecular weight of about 127 to 367.

Example 2

a) Experimental Feeds

Five different feeds are used in the trials. The overall composition of these feeds is given in table 1.

Table 1: Feed composition

	Feed 1 (g/100g)	Feed 2 (g/100g)	Feed 3 (g/100g)	Feed 4 (g/100g)	Feed 5 (g/100g)
Protein source	14.62	14.77	14.78	14.93	15.51
Carbohydrate	64.7	64.4	64.1	63.4	65.4
Lipids	8.21	8.27	8.26	8.37	7.90
Minerals	6.18	5.72	5.69	5.07	6.38
Ash	2.98	3.00	3.00	3.04	2.87

The values are quoted as g of component per 100g of dry matter. The energy provided by all feeds is from 405.9 to 412.5 kCal/100 g. The protein sources used in each feed are different and are as given in table 2.

Table 2 - Protein Source for feeds

Feed	Protein Source
1	whole protein from example 1
2	Hydrolysate 1 from example 1
3	Hydrolysate 2 from example 1
4	Hydrolysate 3 from example 1
5	Free amino acids

The amino acid composition of each protein source is given in table 3.

Table 3 - Amino Acid Composition of Protein Source

Amino Acid	Feed 1 (g/100g)	Feed 2 (g/100g)	Feed 3 (g/100g)	Feed 4 (g/100g)	Feed 5 (g/100g)
Asp	1.00	1.01	1.01	1.02	1.06
Asn	0.66	0.67	0.67	0.68	0.71
Thr	0.77	0.78	0.78	0.79	0.82
Ser	0.72	0.73	0.73	0.73	0.76
Glu	1.60	1.62	1.62	1.64	1.70
Gln	0.86	0.87	0.87	0.88	0.91
Pro	0.60	0.61	0.61	0.62	0.64
Gly	0.29	0.30	0.30	0.30	0.31
Ala	0.68	0.69	0.69	0.70	0.72
Val	0.71	0.72	0.72	0.72	0.75
Cys[1]	0.43	0.43	0.43	0.43	0.45
Met	0.29	0.30	0.30	0.30	0.31
Ile	0.74	0.74	0.74	0.75	0.78
Leu	1.80	1.82	1.82	1.84	1.91
Tyr	0.51	0.51	0.51	0.52	0.54
Phe	0.53	0.54	0.54	0.54	0.56
Lys	1.43	1.44	1.44	1.46	1.51
His	0.27	0.28	0.28	0.28	0.29
Arg	0.42	0.42	0.42	0.43	0.44
Trp	0.31	0.31	0.31	0.32	0.33
Total amino acid	14.62	14.77	14.78	14.93	15.51

(1) in the form of cystine for Feed 5.

b) Experimental Protocol and weight determination

A group of 40 male, Sprague Dawley rats, supplied by IFFA CREDO of l'Arbresle, France are used. The rats have an average weight of 82.3 g ± 1.0. The rats are lodged in separate cages which are illuminated from 7.30 am to 7

pm. For a few days prior to the trials, the rats are allowed to feed *ad libitum* on a standard, pelleted food. The rats have free access to water.

Two days prior to the trials, the rats are separated into 5 groups of 8 rats. Each group of rats is fed, *ad libitum*, one of feeds 1 to 5 for an adaptation period of 2 days. Starting with the first day of the trial, each day at 3 am, 7 am, 11 am, 3 pm, 7 pm and 11 pm, the rats of each group are then fed a precisely metered amount of the feed which they had received during the adaptation period. The feeding regime is continued for a period of 15 days. Each rat is weighed each day.

On the 16th day, a radioactive mixture is prepared by adding 1.7 g of a solution containing ^{14}C Phe (170 µCi per 2.9 µmol) to 9.3 g of a solution containing 30.2 g/l Phe (non radioactive). Half of the rats in each group are anaesthetised and slaughtered at 1.30 pm; the other half are anaesthetised and slaughtered at 3 pm. About 20 minutes prior to being slaughtered, the radioactive mixture is injected intravenously into each rat at 2 ml/200g of body weight.

After slaughter, the blood of each rat is removed and immediately centrifuged at 4°C. The plasma is then separated into fractions and the fractions stored at -20°C. The liver of each rat is rapidly removed, washed with a cold solution containing 9% NaCl, dried, weighed, and frozen in liquid nitrogen. The intestine of each rat is removed, emptied and rinsed in a 5% solution of trichloroacetic acid and separated into three fractions; the whole duodenum, about 13 cm of the jejunum and the remainder of the intestine. Each fraction is dried, weighed, and frozen in liquid nitrogen. The fractions are ground in the liquid nitrogen prior to use. The stomach is removed and weighed before and after emptying. The muscles (gastrocnemius, soleus, and extensor digitorum longus) are removed, de-fatted, washed, dried, weighed and frozen in liquid nitrogen.

The mean weight of the rats at slaughter and the mean weight of the body parts are as follows:

		Feed 1	Feed 2	Feed 3	Feed 4	Feed 5
Body weight/g		173.9	159.4	164.2	154.1	157.7
Stomach	Absolute g	1.19	1.11	1.32	1.25	1.32
	Relative %	0.68	0.70	0.78	0.80	0.84
Intestine	Absolute g	5.95	5.51	5.66	6.02	5.65
	Relative %	3.42	3.46	3.44	3.90	3.59
Duodenum	Absolute g	0.40	0.34	0.34	0.36	0.38
	Relative %	0.23	0.21	0.21	0.24	0.24
Jejunum	Absolute g	1.13	1.15	1.24	1.21	1.24
	Relative %	0.65	0.73	0.76	0.78	0.79
Liver	Absolute g	8.59	8.40	7.87	7.37	7.29
	Relative %	4.93	5.24	4.77	4.80	4.62
Gastrocnemius						
	Absolute g	0.96	0.96	1.04	0.95	0.87
	Relative %	0.55	0.60	0.64	0.62	0.55
Soleus	Absolute g	0.069	0.066	0.068	0.064	0.060
	Relative %	0.040	0.041	0.042	0.042	0.038
Extensor	Absolute g	0.075	0.075	0.072	0.070	0.067
	Relative %	0.043	0.047	0.044	0.046	0.042

The absolute value is the weight of the organ in grams. The relative value is a percentage based on the weight of the organ per 100g of body weight.

The rats fed with Feeds 1 and 3 have a noticeably higher body weight than those feed with the other feeds. The relative weight of the whole intestine of the rats is significantly ($P<0.05$) higher with Feed 4 than the other feeds. Also, the relative weight of the jejunum of the rats is significantly ($P<0.05$) higher with Feeds 3, 4 and 5 than the other feeds. The weight of the liver of the rats fed with Feed 5 is significantly lower ($P<0.05$) than with the other feeds.

c) Determination of protein and RNA content

Tissue samples of 1 g of muscle, 1 g of liver, and 0.5 to 1 g intestine are extracted with a cold solution of 10% trichloroacetic acid to dissolve free amino acids and precipitate protein. The extract is centrifuged at 4°C and 10000 g for

15 minutes. The residue is extracted with a cold solution of 10% trichloroacetic acid and centrifuged at 4°C and 10000 g for 15 minutes. The extraction and centrifuging procedure is repeated again. The residue is collected and the three filtrates are combined.

A column containing 3 ml of Amberlite resin (AG 50 X8, 100-200 mesh) in H^+ form is neutralised to fix free amino acids. The combined filtrate is added to the column and the column washed three times with distilled water. Free amino acids are then eluted using 4N ammonia. The eluant is collected and the ammonia removed by evaporation under vacuum at 40°C. The dry residue is rehydrated to provide a first amino acid solution having a Phe concentration in the region of about 1 µmol/ml. The pH of the first amino acid solution is adjusted to 7.

The residue from the centrifuge is extracted with cold 0.2 M perchloric acid and the extract is centrifuged at 4°C and 10000 g for 15 minutes. The residue is again extracted with perchloric acid and centrifuged in the same manner. The residue is then rehydrated in a solution of water and 3N NaOH. An aliquot of the rehydrated residue is collected for quantification of the protein using the bicinchoninic acid method described in Smith *et al*; 1985; Anal. Biochem., 150, 76-85.

An amount of 3N perchloric acid is added to the remaining rehydrated residue to precipitate protein. The solution is the centrifuged at 4°C at 15000 g for 10 minutes. The RNA concentration in the filtrate is then determined by spectrophotometry (λ_1=232 and λ_2=260) using the technique of Baillie A.G.S and Garlick, P.J.; 1991; Am. J. Physiol., 260, E891-E896.

The residue from the centrifuge is hydrolysed using 5.5N HCl at 150°C over 24 hours to liberate Phe. The HCl is evaporated under vacuum and the residue is rehydrated with water to provide a second amino acid solution having a Phe concentration of about 1 µmol/ml. The pH of the second amino acid solution is adjusted to 7.

d) Determination of Phe radioactivity and rate of Protein synthesis

A Kontron HPLC and Kontron U.V. spectrophotometer (λ = 330nm) are used to separate and detect amino acids. Two solvents are used for the mobile phase:

- solvent A comprising 69g of NaH_2PO_4. H_2O, 32 ml of H_3PO_4, 40g of NaOH particles, a 30% NaOH solution to adjust the pH to 7, and ultra pure water to adjust the volume to 5 litres.

- solvent B comprising 200 ml of solvent A diluted with 300 ml of ultra pure water, 350 ml of methanol, and 150 ml of acetonitrile.

i) Radioactivity of free Phe

The HPLC column is fitted with a Spherisorb OD2 column (5 µm, 250 x 4.6mm) and is operated at 25°C. An orthophthaldialdehyde solution is added to the first amino acid solution in a mass ratio of 2:1 and the mixture is allowed to react. An amount of 40µl of the mixture is added to the column. The column is eluted with solvents A and B at various times; the total amount being added at each time being 1 ml/min. The ratio of solvent A to solvent B at each time is 100:0 at time 0, 40:60 after 7 minutes, 27:73 after 22 minutes, 0:100 after 24 minutes, 0:100 after 26 minutes, and 100:0 after 28 minutes. Elution is continued for 33 minutes. The eluant is collected in fractions of 0.5 ml. Standard solutions containing a mixture of 20 amino acids, including ^{14}C-Phe, are also run through the column to identify and standardise the amino acid peaks.

The fractions of the first amino acid solution containing the Phe peak are collected and the volume adjusted to 5 ml with ultra pure water. Ten ml of a scintillant (Quick Safe A) is added to the solution. The radioactivity is then determined using a Kontron scintillation detector.

ii) Radioactivity of bound Phe

The HPLC column is fitted with a Spherisorb OD2 column (5 µm, 250 x 10mm) and is operated at 25°C. An orthophthaldialdehyde solution is added to the second amino acid solution in a mass ratio of 1:1 and the mixture is allowed to react. An amount of 800µl of the mixture is added to the column. The column is eluted with solvents A and B at various times; the total amount being added at each time being 4.25 ml/min. The ratio of solvent A to solvent B at each time is 100:0 at time 0, 50:50 after 7 minutes, 34:66 after 27 minutes, 0:100 after 29 minutes, 0:100 after 32 minutes, and 100:0 after 34 minutes. Elution is continued for 39 minutes. The eluant is collected in fractions of 0.5 ml.

Standard solutions containing a mixture of 20 amino acids, including ^{14}C-Phe, are also run through the column to identify and standardise the amino acid peaks.

The fractions of the second amino acid solution containing the Phe peak are collected and the volume adjusted to 5 ml with ultra pure water. Ten ml of a scintillant (OPTI Phase "Hisafe") is added to the solution. The radioactivity is then determined using a Kontron scintillation detector.

iii) Calculation of the rate of protein synthesis

A measure of the rate of protein synthesis may be obtained using the formula (McNurlan *et al*; 1979; Biochem. J. 178, 373-379):

Rate of synthesis (% per day) = $S_B/(S_A.t)$

Where S_B is the specific radioactivity value (dpm/μmol) of the bound Phe; S_A is the mean specific radioactivity value (dpm/μmol) of the free Phe; and t is the time in days.

e) Protein and RNA content and rate of protein synthesis.

The protein and RNA content of the different organs and the rate of protein synthesis in the different organs are as follows for each feed:

Liver	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5
Protein Concentration					
mg/g tissue	139.9	160.9	156.4	165.2	147.2
g/100g rat	0.69	0.84	0.75	0.79	0.68
Total protein, g	1.20	1.34	1.24	1.23	1.07
RNA Concentration					
mg/g tissue	7.89	8.10	8.00	8.20	8.17
mg/100g rat	38.8	42.4	38.1	38.9	37.7
Total RNA, mg	67.47	67.93	62.73	60.43	59.45
Protein Synthesis Capacity (mg RNA/g protein)	56.46	51.86	51.76	50.62	56.62
Protein Synthesis Rate (% per day)	82.29	86.94	85.55	89.68	91.53
Daily Protein Synthesis (g protein/day)	1.01	1.18	1.08	1.16	1.01
Ribosomal Efficacy (mg protein/day.mgRNA)	14.69	17.53	17.00	18.50	16.85

No significant difference between the effects that the feeds have on the liver are noticed. Feed 2 however appears to provide a larger relative concentration of protein and RNA in the liver.

Intestine (Duodenum)	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5
Protein Concentration					
mg/g tissue	178.6	180.9	165.0	191.4	162.0
g/100g rat	41.9	39.1	34.2	46.4	41.1
Total protein, g	72.0	61.8	56.0	71.3	65.0
RNA Concentration					
mg/g tissue	8.32	7.97	6.55	8.43	7.59
mg/100g rat	1.98	1.64	1.36	2.05	1.92
Total RNA, mg	3.39	2.59	2.21	3.12	3.03
Protein Synthesis Capacity (mg RNA/g protein)	46.23	44.46	39.69	44.23	48.03
Protein Synthesis Rate (% per day)	97.9	105.7	100.3	135.9	108.0
Daily Protein Synthesis (g protein/day)	72.99	68.52	57.01	97.89	74.44
Ribosomal Efficacy (mg protein/day.mgRNA)	21.2	25.7	25.3	30.8	23.0

Feed 4 has a significant effect ($P < 0.05$) on the protein synthesis rate in the duodenum as compared to the other feeds. Protein and RNA concentrations are raised but not significantly. Further the daily protein synthesis and the ribosomal efficacy are all raised.

Intestine (Jejunum)	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5
Protein Concentration					
mg/g tissue	109.9	109.5	112.2	106.5	110.0
g/100g rat	71.7	79.6	84.1	83.8	87.1
Total protein, g	124.8	126.3	138.7	130.2	137.2
RNA Concentration					
mg/g tissue	7.56	7.39	7.55	7.36	7.87
mg/100g rat	4.90	5.38	5.71	5.82	6.23
Total RNA, mg	8.49	8.50	9.34	8.98	9.79
Protein Synthesis Capacity (mg RNA/g protein)	70.35	67.48	68.31	69.58	71.70
Protein Synthesis Rate (% per day)	89.9	88.4	103.8	111.8	109.5
Daily Protein Synthesis (g protein/day)	111.6	111.8	141.4	144.9	147.7
Ribosomal Efficacy (mg protein/day.mgRNA)	13.02	13.25	15.15	16.14	15.24

The rate of protein synthesis, the daily protein synthesis, and the ribosomal efficacy are significantly ($P<0.05$) raised with feeds 3 to 5 as compared to feeds 1 and 2.

Muscle (Gastrocnemius)	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5
Protein Concentration					
mg/g tissue	189.0	183.2	189.8	180.7	182.4
g/100g rat	109.3	109.7	113.2	108.4	99.9
Total protein, g	188.2	174.7	185.1	166.4	157.4
RNA Concentration					
mg/g tissue	2.14	2.18	1.97	1.91	1.95
mg/100g rat	1.24	1.31	1.18	1.15	1.07
Total RNA, mg	2.14	2.10	1.92	1.77	1.69
Protein Synthesis Capacity (mg RNA/g protein)	11.31	11.94	10.36	10.59	10.84
Protein Synthesis Rate (% per day)	11.00	9.87	9.53	8.83	11.26
Daily Protein Synthesis (g protein/day)	20.75	17.33	18.01	15.38	17.58
Ribosomal Efficacy (mg protein/day.mgRNA)	9.69	8.57	9.17	8.24	10.33

No significant differences are determined. However the daily protein synthesis is reduced with feed 4 when compared to feeds 1 and 5.

Claims

1. Use of a selected form of dietary protein which increases protein concentration or rate of protein synthesis in a selected organ as a protein source in the preparation of a nutritional formula for promoting the growth or recovery of the specific organ in a mammal.

2. Use according to claim 1 in which the dietary protein is a protein hydrolysate having a degree of hydrolysis of at least about 30% for the preparation of a nutritional formula for increasing protein concentration and synthesis in the small intestine.

3. Use according to claim 2 formula for the preparation of a nutritional formula for increasing protein concentration and synthesis in the duodenum.

4 Use according to claim 2 or claim 3 in which the protein hydrolysate comprises more than about 30% by weight of di- and tri-peptides and has a non protein nitrogen concentration of at least about 85% of total nitrogen.

5. Use according to claim 1 in which the dietary protein is (i) a protein hydrolysate having a degree of hydrolysis of at least about 15%; (ii) free amino acids; or (iii) mixtures thereof, for the preparation of a nutritional formula for increasing protein concentration and synthesis in the jejunum.

6. Use according to claim 5 in which the dietary protein is a protein hydrolysate which comprises more than about 20% by weight of di- and tri-peptides and which has a non protein nitrogen concentration of at least about 60% of total nitrogen.

7. Use according to claim 1 in which the dietary protein is in the form of free amino acids for the preparation of a nutritional formula for maintaining muscle protein synthesis and for the prophylaxis or treatment of muscular atrophy.

8. Use according to claim 7 for the preparation of a nutritional formula for mammals having compromised gut function.

9. Use according to claim 1 in which the dietary protein is a protein hydrolysate for the preparation of a nutritional formula for increasing protein concentration and synthesis in underdeveloped intestines of premature babies.

10. Use according to claim 9 in which the protein hydrolysate comprises more than about 30% by weight of di- and tri-peptides and has a non protein nitrogen concentration of at least about 85% of total nitrogen.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/05843

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L1/305 A61K31/195 A23K1/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 9202 Derwent Publications Ltd., London, GB; Class B05, AN 92-013563 XP002058210 -& JP 03 264525 A (OTSUKA SEIYAKU KYOG) , 25 November 1991 see abstract	1
X A	EP 0 189 161 A (ABBOTT) 30 July 1986 see page 1, line 7-28 see page 3, line 13-34 see claims 1,3,4	1 2-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 January 1999	02/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van Moer, A

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/05843

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 221 668 A (M.F.HENNINGFIELD ET AL.) 22 June 1993	1
A	see column 1, line 59 - column 2, line 15 see column 2, line 38-46 see column 10, line 18-45 see claims 1,10,11,18	2-10
X	EP 0 704 218 A (SNOW BRAND) 3 April 1996 see page 3, line 45-48; claims	1
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A	EP 0 017 867 A (MAX-PLANCK-GESELLSCHAFT) 29 October 1980 cited in the application see the whole document	1-10
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INTERNATIONAL SEARCH REPORT

Ir tional Application No
PCT/EP 98/05843

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
NO 5486/WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 98/ 05843	14/09/1998	16/09/1997
Applicant		
SOCIETE DES PRODUITS NESTLE S.A. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. ---
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/05843

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L1/305 A61K31/195 A23K1/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 9202 Derwent Publications Ltd., London, GB; Class B05, AN 92-013563 XP002058210 -& JP 03 264525 A (OTSUKA SEIYAKU KYOG) , 25 November 1991 see abstract	1
X A	EP 0 189 161 A (ABBOTT) 30 July 1986 see page 1, line 7-28 see page 3, line 13-34 see claims 1,3,4	1 2-10

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 January 1999	02/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van Moer, A

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/05843

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X A	US 5 221 668 A (M.F.HENNINGFIELD ET AL.) 22 June 1993 see column 1, line 59 - column 2, line 15 see column 2, line 38-46 see column 10, line 18-45 see claims 1,10,11,18	1 2-10
X	EP 0 704 218 A (SNOW BRAND) 3 April 1996 see page 3, line 45-48; claims	1
A	US 5 580 903 A (K.MAWATARI ET AL) 3 December 1996 cited in the application see claims & JP 05 229940 A	1-10
X	WO 92 20707 A (MERRELL DOW PHARMACEUTICALS) 26 November 1992 cited in the application see claims 1,7	1
A	EP 0 017 867 A (MAX-PLANCK-GESELLSCHAFT) 29 October 1980 cited in the application see the whole document	1-10
A	EP 0 322 589 A (NESTLE) 5 July 1989 cited in the application see the whole document	1
P,X	WO 97 39641 A (M.D.FOODS) 30 October 1997 see claims	1

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 98/05843

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 189161	A	30-07-1986	AU	587414 B	17-08-1989
			AU	5255186 A	07-08-1986
			CA	1271360 A	10-07-1990
			DK	41586 A	30-07-1986
			GR	860211 A	26-05-1986
			IE	58454 B	22-09-1993
			JP	2050529 C	10-05-1996
			JP	7072127 B	02-08-1995
			JP	61180715 A	13-08-1986
			US	4670268 A	02-06-1987
US 5221668	A	22-06-1993	AU	3614393 A	13-09-1993
			CA	2128078 A	02-09-1993
			EP	0630181 A	28-12-1994
			JP	2644086 B	25-08-1997
			JP	7500348 T	12-01-1995
			MX	9300999 A	01-09-1993
			NZ	249392 A	26-01-1996
			WO	9316595 A	02-09-1993
EP 704218	A	03-04-1996	JP	8151331 A	11-06-1996
US 5580903	A	03-12-1996	JP	5229940 A	07-09-1993
WO 9220707	A	26-11-1992	AT	156492 T	15-08-1997
			AU	659659 B	25-05-1995
			AU	2184092 A	30-12-1992
			CA	2109322 A	24-11-1992
			DE	69221486 D	11-09-1997
			DE	69221486 T	19-03-1998
			DK	585397 T	25-08-1997
			EP	0585397 A	09-03-1994
			ES	2106188 T	01-11-1997
			GR	3025003 T	30-01-1998
			JP	6508126 T	14-09-1994
			US	5428019 A	27-06-1995
EP 17867	A	29-10-1980	DE	2914903 B	23-10-1980
			JP	55143915 A	10-11-1980
			US	4330528 A	18-05-1982
EP 322589	A	05-07-1989	EP	0321603 A	28-06-1989
			AU	2659688 A	29-06-1989
			CA	1334064 A	24-01-1995
			DE	3877733 A	04-03-1993
			ES	2053690 T	01-08-1994
			JP	2002319 A	08-01-1990
			JP	6077504 B	05-10-1994
			MX	169602 B	14-07-1993
			PH	26140 A	18-03-1992
			PT	89325 A,B	29-12-1989
			US	5039532 A	13-08-1991
WO 9739641	A	30-10-1997	AU	2633997 A	12-11-1997

XP-002058210

1/1 - (C) WPI / DERWENT
AN - 92-013563 ç13!
AP - JP900064606 900314
PR - JP900064606 900314
TI - Aminoacid transfusion solns. - contain free aminoacid(s) and di:peptide(s) of glutamine, used by living bodies to maintain intestinal tract functions, etc.
IW - AMINOACID TRANSFUSION SOLUTION CONTAIN FREE AMINOACID DI PEPTIDE GLUTAMINE LIVE BODY MAINTAIN INTESTINAL TRACT FUNCTION
PA - (SAKA) OTSUKA SEIYAKU KYOG
PN - JP3264525 A 911125 DW9202 000pp
ORD - 1991-11-25
IC - A61K31/19
FS - CPI
DC - B05
AB - J03264525 Solns. contain free aminoacids and dipeptides of glutamine in given compsn. range. The dipeptides are one or more of L-alanyl-L-glutamine, L-glutamyl-N-alanine, glycyl-L-glutamine and L-glutamyl-Glycine. The free converted amt. of glutamine is 10-50 w/w% of the total aminoacid amt. The sum of branched chain aminoacids and free converted glutamine is 30-70 w/w% of the total aminoacid amt.
- L-Aminoacid and dipeptide compsn. ranges in (mg/dl) are Leu 1000-2000; Ile 500-1600; Val 500-1600; Lys 800-1600; Thr 400-700; Try 100-300; Met 300-800; Phe 600-1000; Arg 500-2500; His 200-800; Ala 0-2000; Gly 0-1000; Pro 0-1000; Ser 0-500; Cys 0-200; Tyr 0-200; Asp 0-300; Glu 0-300; and Dipeptides of glu 1000-20000
- USE/ADVANTAGE - The transfusion solns. are nutrients stable in aq. soln. and contain high ratios of dipeptides of glutamine and branched chain amino acids which are readily utilised by living bodies under attacks of foreign matters. The elevated concn. of total amino acids can maintain and improve intestinal tract functions which are vulnerable to attacks from outside. The solns. can also prevent catabolism of muscular proteins and accelerate synthesis of tissue proteins. A small amt. of them can be used as transfusion solns. with excellent nutrition and high calorie. Excessive burdens to the kidney can be avoided.
- (8pp Dwg.No.0/0)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/05843

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L1/305 A61K31/195 A23K1/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 9202 Derwent Publications Ltd., London, GB; Class B05, AN 92-013563 XP002058210 -& JP 03 264525 A (OTSUKA SEIYAKU KYOG) , 25 November 1991 see abstract ---	1
X A	EP 0 189 161 A (ABBOTT) 30 July 1986 see page 1, line 7-28 see page 3, line 13-34 see claims 1,3,4 --- -/--	1 2-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 January 1999	02/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van Moer, A

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/05843

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 221 668 A (M.F.HENNINGFIELD ET AL.) 22 June 1993	1
A	see column 1, line 59 - column 2, line 15 see column 2, line 38-46 see column 10, line 18-45 see claims 1,10,11,18	2-10
X	EP 0 704 218 A (SNOW BRAND) 3 April 1996 see page 3, line 45-48; claims	1
A	US 5 580 903 A (K.MAWATARI ET AL) 3 December 1996 cited in the application see claims & JP 05 229940 A	1-10
X	WO 92 20707 A (MERRELL DOW PHARMACEUTICALS) 26 November 1992 cited in the application see claims 1,7	1
A	EP 0 017 867 A (MAX-PLANCK-GESELLSCHAFT) 29 October 1980 cited in the application see the whole document	1-10
A	EP 0 322 589 A (NESTLE) 5 July 1989 cited in the application see the whole document	1
P,X	WO 97 39641 A (M.D.FOODS) 30 October 1997 see claims	1

1

INTERNATIONAL SEARCH REPORT

Ir itional Application No
PCT/EP 98/05843

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 189161 A	30-07-1986	AU 587414 B	17-08-1989
		AU 5255186 A	07-08-1986
		CA 1271360 A	10-07-1990
		DK 41586 A	30-07-1986
		GR 860211 A	26-05-1986
		IE 58454 B	22-09-1993
		JP 2050529 C	10-05-1996
		JP 7072127 B	02-08-1995
		JP 61180715 A	13-08-1986
		US 4670268 A	02-06-1987
US 5221668 A	22-06-1993	AU 3614393 A	13-09-1993
		CA 2128078 A	02-09-1993
		EP 0630181 A	28-12-1994
		JP 2644086 B	25-08-1997
		JP 7500348 T	12-01-1995
		MX 9300999 A	01-09-1993
		NZ 249392 A	26-01-1996
		WO 9316595 A	02-09-1993
EP 704218 A	03-04-1996	JP 8151331 A	11-06-1996
US 5580903 A	03-12-1996	JP 5229940 A	07-09-1993
WO 9220707 A	26-11-1992	AT 156492 T	15-08-1997
		AU 659659 B	25-05-1995
		AU 2184092 A	30-12-1992
		CA 2109322 A	24-11-1992
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		DE 69221486 T	19-03-1998
		DK 585397 T	25-08-1997
		EP 0585397 A	09-03-1994
		ES 2106188 T	01-11-1997
		GR 3025003 T	30-01-1998
		JP 6508126 T	14-09-1994
		US 5428019 A	27-06-1995
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		JP 55143915 A	10-11-1980
		US 4330528 A	18-05-1982
EP 322589 A	05-07-1989	EP 0321603 A	28-06-1989
		AU 2659688 A	29-06-1989
		CA 1334064 A	24-01-1995
		DE 3877733 A	04-03-1993
		ES 2053690 T	01-08-1994
		JP 2002319 A	08-01-1990
		JP 6077504 B	05-10-1994
		MX 169602 B	14-07-1993
		PH 26140 A	18-03-1992
		PT 89325 A,B	29-12-1989
		US 5039532 A	13-08-1991
WO 9739641 A	30-10-1997	AU 2633997 A	12-11-1997

PATENT COOPERATION TREATY

GL

Seen GL 7/12/99

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

McCONNELL Bruce
SOCIETE DES PRODUITS NESTLE S.A.
Case Postale 353
CH-1800 Vevey
SUISSE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	03. 12. 99
Applicant's or agent's file reference: NO 5486/WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/05843	14/09/1998	16/09/1997

Applicant
SOCIETE DES PRODUITS NESTLE S.A. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Bleeker, M Tel.+49 89 2399-8141

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
NO 5486/WO		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/05843	14/09/1998	16/09/1997

International Patent Classification (IPC) or national classification and IPC
A23L1/305

Applicant
SOCIETE DES PRODUITS NESTLE S.A. et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12/04/1999	03. 12. 99

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

Heirbaut, M

Telephone No. +49 89 2399 8642

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/05843

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-24 as originally filed

Claims, No.:

1-10 as received on 18/09/1999 with letter of 16/09/1999

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-10
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

V

1. Reference is made to the following documents (D):

D1= DATABASE WPI, Section Ch, Week 9202, Derwent Publications Ltd., London, GB; Class B05, AN 92-013563, XP002058210 & JP-A-3 264 525, 25 November 1991
D2= EP-A-0 189 161
D3= US-5 221 668
D4= EP-A-0 704 218
D5= US-5 580 903
D6= WO-A-9 220 707
D7= EP-A-0 017 867
D8= WO-A-9 739 641

2. The subject-matter of present independent claim 1 (use) does not meet the requirements of novelty (Article 33(2) PCT) in the light of any of the prior art documents D1-D7, which describe the combination of features disclosed in said claim.

Document D1 describes transfusion solutions having "excellent nutrition and high calorie", containing high ratios of dipeptides of glutamine and branched chain amino acids, and can maintain and improve intestinal tract functions, and can prevent catabolism of muscular proteins and accelerate synthesis of tissue proteins (see in particular abstract of D1).

Document D2 describes hypoallergenic compositions comprising protein hydrolysates such as casein and whey hydrolysate, which comprise short peptide fragments and/or free amino acids instead of intact protein, as a major source of nitrogen, as well as carbohydrates, vitamins, minerals and supplemented amino acids to upgrade their nutritional quality (see in particular claims 1, 3 and 8; page 1, lines 7-23 of D2). Said hypoallergenic compositions are often medically used in the treatment of cystic fibrosis, chronic diarrhea, galactosaemia, small bowel resection, steatorrhoea and protein-calorie malnutrition (see in particular page 1, lines 26-28 of D2). These are conditions also described in the present application

(see in particular page 8, lines 17-23 of the present description).

Document D3 describes a liquid nutritional product comprising a protein system comprising by weight about 20-30% of lactalbumin hydrolysate, about 60-70% partially hydrolysed sodium caseinate and about 8-14% L-arginine, supplemental L-arginine providing about 1-3% of the total calories in the product (see in particular claim 1 of D3). Accelerated wound healing and nitrogen retention after injury have been attributed to the feeding of arginine (see in particular column 10, lines 43-46 of D3). Furthermore, it is described that appropriate enteral nutrition following injury may minimize malnutrition, provide nutrients to the immune system and maintain the gut epithelial which acts as a barrier to translocation of bacteria, which may help prevent the development of sepsis (see in particular column 2, lines 11-16 of D3).

Document D4 describes a food or drink composition comprising a basic protein fraction derived from milk or a basic peptide fraction obtained by hydrolysing a basic protein fraction derived from milk, as a bone reinforcing agent (see in particular claims 1, 5 and 10 of D4). Said bone reinforcing agent promotes the growth of osteoblasts and suppresses the resorption of osteoblasts, thereby strengthening bone without causing side effects (see in particular page 2, lines 43-45 of D4).

Document D5 describes a liver regeneration accelerator comprising alanine, glutamine or di- or tripeptides comprising both amino acids (see in particular column 2, lines 49-56 of D5). After administration of said liver regeneration accelerator to rats, the liver labelling index rose and liver wet weight increased (see in particular column 2, lines 35-40 of D5).

Document D6 describes that bombesin elicts cell mitogenic responses in a number of tissues, e.g. Swiss 3T3 murine embryonal fibroblasts, growth-arrested ocular vesicles, gastrin cells in the antral mucosa of the rat stomach (see in particular page 27, lines 9-30 of D5). A peptide derivative of bombesin is used in the manufacture of a medicament for stimulating growth of organ tissues of the lung, pancreas or intestine in a patient in need thereof (see in particular claim 7 of D6).

Document D7 describes a medicament for stimulation of the proliferation of liver cells, comprising as an active agent a blood plasma extract with a molecular weight of about 1200 D, obtainable from blood plasma by acidification to pH 5.5, heat denaturation at about 95°C, centrifugation and reaction with peptide/peptidylhydrolase (see in particular claim 1 of D7).

The protein hydrolysates, peptides and amino acids described in documents D1-D7 are considered to represent dietary protein and describe the technical effect of promotion of growth or recovery of a specific organ in a mammal.

3. Document D8, with filing date 18.04.1997, priority of 18.04.1996 claimed, was published on 30.10.1997. A decision whether the priority of 16.09.1997 has been validly claimed by the Applicant was not possible, as the priority document was not available for examination. Under the condition that the priority of 18.04.1996 is validly claimed, document D8 does not constitute prior art for the present application (Rule 64.1(b) PCT). Document D8 describes an energy supplement comprising a protein hydrolysate having a degree of hydrolysis of 1-50 (considered to represent dietary protein), which reduces muscle breakdown during metabolic stress and allows for faster rebuilding of degraded muscular proteins after metabolic stress (see in particular claims 2 and 8; page 2, lines 7-24 of D8). Useful protein hydrolysates are e.g. whey and casein protein hydrolysates (see in particular claim 12; page 2, lines 28-32 of D8).

VII

1. The present application does not meet the requirements of Rule 5.1 (a)(ii) PCT, as the prior art documents D1-D5 and D8 have not been cited in the description (see also PCT Guidelines II, 4.4).

VIII

1. Present independent claim 1 does not meet the requirements of Article 6 PCT in that the subject-matter for which protection is sought is not clearly defined. The claim attempts to define the dietary protein in terms of the effects of its use rather

than in terms of its structural features (e.g. degree of hydrolysis, molecular weight) (PCT Guidelines C-III, 4.7.).

2. The passages on page 2, lines 30-34, page 3, lines 18-21 and 30-35, page 4, lines 12-17 of the present description, stating that methods of medical treatment are embodiments of the invention, do not meet the requirements of Article 34.4 (a)(i) PCT and Rule 67.1 (iv) PCT.

Claims

1. Use of a selected form of dietary protein which increases protein concentration or rate of protein synthesis in a selected organ as a protein source in the preparation of a nutritional formula for promoting the growth or recovery of the specific organ in a mammal.

2. Use according to claim 1 in which the dietary protein is a protein hydrolysate having a degree of hydrolysis of at least about 30% for the preparation of a nutritional formula for increasing protein concentration and synthesis in the small intestine.

3. Use according to claim 2 in which the dietary protein is used in the preparation of a nutritional formula for increasing protein concentration and synthesis in the duodenum.

4 Use according to claim 2 or claim 3 in which the protein hydrolysate comprises more than about 30% by weight of di- and tri-peptides and has a non protein nitrogen concentration of at least about 85% of total nitrogen.

5. Use according to claim 1 in which the dietary protein is (i) a protein hydrolysate having a degree of hydrolysis of at least about 15%; (ii) free amino acids; or (iii) mixtures thereof, for the preparation of a nutritional formula for increasing protein concentration and synthesis in the jejunum.

6. Use according to claim 5 in which the dietary protein is a protein hydrolysate which comprises more than about 20% by weight of di- and tri-peptides and which has a non protein nitrogen concentration of at least about 60% of total nitrogen.

7. Use according to claim 1 in which the dietary protein is in the form of free amino acids for the preparation of a nutritional formula for maintaining muscle protein synthesis and for the prophylaxis or treatment of muscular atrophy.

8. Use according to claim 7 in which the dietary protein is used in the preparation of a nutritional formula for mammals having compromised gut function.

9. Use according to claim 1 in which the dietary protein is a protein hydrolysate for the preparation of a nutritional formula for increasing protein concentration and synthesis in underdeveloped intestines of premature babies.

10. Use according to claim 9 in which the protein hydrolysate comprises more than about 30% by weight of di- and tri-peptides and has a non protein nitrogen concentration of at least about 85% of total nitrogen.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
SOCIETE DES PRODUITS NESTLE S.A.
Case Postale 353
CH-1800 Vevey
SWITZERLAND

05 FEV. 1999

Applicant's or agent's file reference NO 5486/WO	Date of mailing *(day/month/year)* 02/02/1999 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/EP 98/ 05843	International filing date *(day/month/year)* 14/09/1998
Applicant SOCIETE DES PRODUITS NESTLE S.A. et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicants's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Cristina Iacoponi

Form PCT/ISA/220 (January 1994)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
NO 5486/WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 98/ 05843	14/09/1998	16/09/1997
Applicant		
SOCIETE DES PRODUITS NESTLE S.A. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. ---
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/05843

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L1/305 A61K31/195 A23K1/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 9202 Derwent Publications Ltd., London, GB; Class B05, AN 92-013563 XP002058210 -& JP 03 264525 A (OTSUKA SEIYAKU KYOG) , 25 November 1991 see abstract	1
X A	EP 0 189 161 A (ABBOTT) 30 July 1986 see page 1, line 7-28 see page 3, line 13-34 see claims 1,3,4	1 2-10

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 January 1999	02/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van Moer, A

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/05843

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 221 668 A (M.F.HENNINGFIELD ET AL.) 22 June 1993	1
A	see column 1, line 59 - column 2, line 15 see column 2, line 38-46 see column 10, line 18-45 see claims 1,10,11,18	2-10
X	EP 0 704 218 A (SNOW BRAND) 3 April 1996 see page 3, line 45-48; claims	1
A	US 5 580 903 A (K.MAWATARI ET AL) 3 December 1996 cited in the application see claims & JP 05 229940 A	1-10
X	WO 92 20707 A (MERRELL DOW PHARMACEUTICALS) 26 November 1992 cited in the application see claims 1,7	1
A	EP 0 017 867 A (MAX-PLANCK-GESELLSCHAFT) 29 October 1980 cited in the application see the whole document	1-10
A	EP 0 322 589 A (NESTLE) 5 July 1989 cited in the application see the whole document	1
P,X	WO 97 39641 A (M.D.FOODS) 30 October 1997 see claims	1

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 98/05843

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 189161 A	30-07-1986	AU 587414 B	17-08-1989
		AU 5255186 A	07-08-1986
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		DK 41586 A	30-07-1986
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		MX 169602 B	14-07-1993
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